

Application Serial No. 10/554,288  
Amendment After Final dated 5 March 2008  
Reply to Office Action dated 24 January 2008

*AMENDMENTS TO THE CLAIMS:*

This following listing of claims will replace all prior versions, and listings of claims in the application.

**Listing of Claims:**

Claim 1 (Currently Amended): A method of isolating a  $\beta$  (1-3)  $\beta$  (1-4) glucan from a milled cereal grain or a milled part of the cereal grain, comprising:

(i) extracting the milled cereal grain or the milled part of the cereal grain with an alkaline solution having a value of pH of between 9 to 10 for a period of time of about 15 to about 45 minutes to produce an extract containing at least about 0.4 weight percent  $\beta$  (1-3)  $\beta$  (1-4) glucan;

(ii) removing insoluble material, and removing particulate material having a particle size of greater than about 0.2  $\mu\text{m}$  from said extract to produce a purified extract comprising  $\beta$  (1-3)  $\beta$  (1-4) glucan having a particle size of less than 0.2  $\mu\text{m}$ , wherein the step of removing particulate material comprises:

using microfiltration to filter out material having a particle size of greater than about 0.2  $\mu\text{m}$  from said extract by microfiltration and produce a filtrate comprising  $\beta$  (1-3)  $\beta$  (1-4) glucan having a particle size of less than 0.2  $\mu\text{m}$ ;

(iii) adding from between 10% to 20% (vol/vol) of a  $\text{C}_1\text{-C}_4$  alcohol to the purified extract to precipitate the  $\beta$  (1-3)  $\beta$  (1-4) glucan, and

(iv) isolating the  $\beta$ (1-3)  $\beta$ (1-4) glucan.

Claim 2 (Previously Presented): The method of claim 1, wherein the  $\text{C}_1\text{-C}_4$  alcohol is selected from the group consisting of methanol, ethanol and isopropanol.

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Claim 3 (Previously Presented): The method of claim 2, wherein the C<sub>1</sub>-C<sub>4</sub> alcohol is ethanol.

Claim 4 (Currently Amended): The method of claim 1, wherein, said step of removing particulate material further comprises the following steps prior to the microfiltration step:

one, or more than one step of adding a flocculant, a coagulant or both a flocculant and a coagulant to said extract to coagulate particulate material having a particle size of greater than about 0.2  $\mu\text{m}$ , and removing coagulated material from said extract; and

digesting starch material in said extract, ~~and~~

~~filtering out particulate material having a particle size of greater than about 0.2  $\mu\text{m}$  from said extract to produce a purified extract.~~

Claim 5 (Original): The method of claim 4, wherein, in said step of digesting, said starch material is digested with an enzyme.

Claim 6 (Original): The method of claim 5, wherein prior to digesting said starch material, said alkaline solution is neutralized.

Claim 7 (Original): The method of claim 6, wherein following the digestion of said starch material, said enzyme is inactivated.

Claim 8 (Original): The method of claim 7, wherein said enzyme is inactivated by acidifying the neutralized solution.

Claim 9 (Original): The method of claim 5, wherein said enzyme is an amylase.

Claim 10 (Original): The method of claim 9, wherein said amylase does not require a calcium cofactor.

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Claim 11 (Original): The method of claim 1, wherein the cereal is selected from the group consisting of a cultivar of barley, a cultivar of oat, a cultivar of wheat, a cultivar of rye, a cultivar of sorghum, a cultivar of millet, and a cultivar of corn.

Claims 12-13 (Canceled).

Claim 14 (Original): The method of claim 1, wherein said step of adding (step iii) is conducted at a temperature of from about 1°C to about 10°C.

Claim 15 (Previously Presented): The method of claim 1, further comprising one, or more than one step of dissolving the isolated  $\beta$  (1-3)  $\beta$  (1-4) glucan in an aqueous solution, precipitating the  $\beta$  (1-3)  $\beta$  (1-4) glucan by adding between 10% to 20% (vol/vol) of the C<sub>1</sub>-C<sub>4</sub> alcohol to the aqueous solution, and isolating the  $\beta$  (1-3)  $\beta$  (1-4) glucan.

Claim 16 (Currently Amended): A method of isolating a  $\beta$  (1-3)  $\beta$  (1-4) glucan from a milled cereal grain or a milled part of the cereal grain, comprising:

(i) extracting the milled cereal grain or the milled part of the cereal grain with an alkaline solution having a value of pH of ~~between 9 to 10~~ about 9.25 to about 9.75 for a period of time of about 15 to about 45 minutes to produce an extract comprising at least about 0.4 weight percent  $\beta$  (1-3)  $\beta$  (1-4) glucan;

(ii) removing insoluble material, and removing particulate material having a particle size of greater than about 0.2  $\mu$ m from said extract to produce a purified extract comprising  $\beta$  (1-3)  $\beta$  (1-4) glucan having a particle size of less than 0.2  $\mu$ m, wherein the step of removing particulate material comprises:

~~one, or more than one step of adding a flocculant, selected from the group consisting of a polyacrylamide, a quaternary acrylate salt and a natural flocculant macromolecule, a coagulant, selected from the group consisting of alum, lime, ferric chloride, ferrous sulfate, an organic~~

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~~polymer and a synthetic polyelectrolyte with anionic or cationic functional groups~~, or both the flocculant and the coagulant to said extract to coagulate particulate material having a particle size of greater than about 0.2  $\mu\text{m}$ , and removing coagulated material from said extract;

enzymatically digesting starch material in said extract, and

using microfiltration to filter ~~filtering~~ out particulate material having a particle size of greater than about 0.2  $\mu\text{m}$  from said extract ~~to~~ and produce the purified extract comprising  $\beta$  (1-3)  $\beta$  (1-4) glucan having a particle size of less than 0.2  $\mu\text{m}$  as a filtrate;

(iii) adding about 10% to about 25% (vol/vol) of a  $\text{C}_1\text{-C}_4$  alcohol to the purified extract to precipitate the  $\beta$ (1-3)  $\beta$ (1-4) glucan, and

(iv) isolating the  $\beta$ (1-3)  $\beta$ (1-4) glucan.

Claims 17-27 (Canceled).

Claim 28 (Previously Presented): The method of claim 4, wherein the flocculant is selected from the group consisting of a polyacrylamide, a quaternary acrylate salt and a natural flocculant macromolecule, and the coagulant is selected from the group consisting of alum, lime, ferric chloride, ferrous sulfate, an organic polymer and a synthetic polyelectrolyte with anionic or cationic functional groups.

Claim 29 (Previously Presented): The method of claim 1, wherein about 15% to about 17% (vol/vol) of the  $\text{C}_1\text{-C}_4$  alcohol is added to the purified extract in step (iii).

Claim 30 (Previously Presented): The method of claim 16, wherein about 10% to about 20% (vol/vol) of the  $\text{C}_1\text{-C}_4$  alcohol is added to the purified extract in step (iii).

Claim 31 (Previously Presented): The method of claim 16, wherein about 15% to about 17% (vol/vol) of the  $\text{C}_1\text{-C}_4$  alcohol is added to the purified extract in step (iii).